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Equine grass sickness in Scotland: A case-control study of signalment- and meteorology- related risk factors

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Summary

Reasons for performing study: Equine grass sickness (EGS) remains a frequently fatal disease of equids in Britain. Since previous investigations of signalment- and meteorology-related risk factors for EGS have yielded some conflicting data, further investigation is warranted.

Objectives: To identify signalment- and meteorology- related risk factors for EGS in Scotland.

Methods: A retrospective time-matched case-control study was undertaken using data for 455 EGS cases and 910 time-matched controls that were referred to the Royal Dick School of Veterinary Studies (R(D)SVS), and average UK Meteorological Office weather station meteorological values from the month of admission of the animal, from the 3, 6 and 12 months prior to admission, and for the entire 1990 to 2006 period.

Results and conclusion: Signalment-related risk factors associated with an increased risk of EGS were native Scottish compared to cross-breeds (Odds ratio (OR) = 3.56, 95% Confidence Interval (CI) = 2.43-5.43) and animals living on premises located further north within the study region (OR = 1.08, CI = 1.06-1.10). There was a decreased risk of EGS in animals aged 11-20 years old compared to animals 2-10 years old (OR = 0.32, CI = 0.22-0.45), non-native Scottish pure breeds compared to cross-breeds (OR = 0.71, CI = 0.54-0-94), and stallions compared to mares (OR = 0.43, CI = 0.22-0.86). Meteorology-related risk factors associated with an increased risk of EGS were (if Ordnance Survey northing excluded) more sun hours (OR>1.43) and more frost days (OR>1.13), while there was a decreased risk of EGS with higher average maximum temperature (OR<0.83).

Potential relevance: The signalment-related risk factors will help owners identify high-risk animals, thereby allowing them to prioritise management strategies. The identification of

meteorological risk factors may assist studies on the aetiology of EGS.

Introduction

Equine grass sickness (EGS) is a predominantly fatal neurodegenerative disease of equids [1; 2]. While the aetiology remains unknown, accumulating evidence suggests that EGS is a toxicoinfectious form of botulism, with neurotoxin production by *Clostridium botulinum* occurring within the gastrointestinal tract [3; 4].

Signalment-related risk factors have been identified for EGS, but conflicting data from previous studies indicates that further investigation is warranted [5-7]. EGS affects a wide range of ages (2 months to 47 years) [8], but risk is considered highest in young animals [4; 6; 9]. No consistent breed or sex associations have been identified [4-6].

Great Britain has the highest prevalence of EGS worldwide, with a high proportion of cases occurring in Eastern Scotland; however cases are also reported from every county in England and Wales [10; 11]. As EGS occurs most commonly in grazing horses/ponies [5; 6; 12], it has been proposed that the geographic distribution of EGS is associated with meteorological factors that predispose to disease development by influencing growth of pasture herbage and/or causal environmental microorganisms [13; 14]. Previous investigations of sun hours, temperature, rainfall and frost in the period immediately preceding the occurrence of EGS have yielded conflicting conclusions [6; 7; 14]. Therefore, the aim of this study was to investigate further signalment- and meteorology- related risk factors for EGS, using the largest case-control study of EGS to date.

Materials and Methods

Database of EGS cases

Potential risk factors were investigated using a retrospective case-control study. Cases were any horse/pony diagnosed with EGS by clinicians at the Royal Dick School of Veterinary Studies (R(D)SVS) between 1st January 1990 and 1st June 2006. EGS was confirmed by histopathological examination of autonomic and/or enteric ganglia in all cases except the surviving 84 chronic EGS cases. For each EGS case 2 time-matched controls were identified and these were grazing horses/ponies referred to the R(D)SVS for any reason other than EGS, immediately prior and after the EGS case. Animals with a previous diagnosis of EGS were excluded as controls. Cases or controls originating out of Scotland were excluded. The following data were retrieved: age, sex, breed, and owner's postcode. Each case and control postcode was converted into 6-digit Ordnance Survey (OS) easting and northing grid references (acting as equivalents for longitude and latitude) using the Office for National Statistics (www.ons.gov.uk) Postcode Directory Open edition database (August 2010 download from www.edina.ed.ac.uk).

Signalment-related risk factors investigated were age, sex and breed. Age was examined as a continuous variable in years and as a categorical variable in 0-1, 2-10, 11-20, >20 blocks (a clear reduction in cases in <1 year olds has previously been reported [8]). Sex was examined as: male/female, female/stallion/gelding and colt/filly/gelding/mare/stallion. Breed was considered a categorical variable with native Scottish breeds (Clydesdale, Eriskay, Highland, Shetland), other pure breeds, and cross-breeds (Cob, Hunter, pony, other pure breed crosses).

Meteorological variables

Monthly meteorological data from 1990-2006 were obtained from the UK Meteorological Office (http://www.metoffice.gov.uk) 5 km Gridded Climate Projection datasets. Data on the number of sun hours per month (sun hours); number of days in the month with rain (rain days); rainfall per month (rainfall (mm)); number of days in the month with frost (frost days) and the minimum and maximum temperatures (ºC) observed in a month were selected. A number of average values were extracted from these datasets, namely (a) entire 1990 to 2006 period, (b) month of hospital admission, and (c) 3, 6 and 12 months prior to admission.

Meteorological data were matched to each horse using the OS easting and OS northing associated with the horse.

Data analyses

Standard linear regression of OS easting and northing against meteorological variables were performed. Correlations between meteorological variables were assessed by Spearman rank (ρ_s). For risk factor analyses, univariable conditional logistic regression analyses (hereafter '*univariable conditional*') examined individual associations between cases *versus* controls and variables. Each case and its 2 matched controls were entered as a set. Associations where $P<0.25$ [15] were then entered into a multivariable conditional logistic regression model (hereafter '*multivariable conditional*') and variables excluded until obtaining a final minimal statistically significant model. Conditional logistic regression results are expressed as odds ratios (OR), 95% confidence intervals (95% CI) and Wald chi-square P-values. $P<0.05$ was taken to indicate statistical significance. All analyses were done in R (© 2012 The R foundation for Statistical Computing).

Results

Signalment

The study population comprised 1,365 animals, with 455 cases and 910 matched controls, from the width and length of Scotland (excluding the Outer Hebrides and Shetland) (Fig 1). However, animals were predominantly located in Eastern Scotland, with 98.5% of animals east of OS easting 240000 [4°29'W]. In addition, 99% of animals were south of OS northing 890000 [57°53'N]. Because of this geographical bias 21 animals west of OS easting 240000 were excluded from OS easting analyses and 13 animals north of OS northing 890000 were excluded from OS northing analyses (though this made no qualitative difference to any analyses (not shown)). There was no significant association between EGS and OS easting (P = 0.29), but a highly significant association with OS northing - the odds of being a case increased by 1.08 for every 10 km further north (P<0.001, Table 1). The characteristics of the total study population are presented in Table 1. Most animals (>92%) were adults, with 67.5% aged between 2 and 10 years. Cross breeds comprised 46% of animals, with native Scottish breeds comprising ~14%. Males comprised 56.5% of animals, with mares and geldings the 2 most common sex groups.

The odds of being a case with increasing age were not linear (Fig 2). The percentage of cases peaked at 2 years, then declined before an apparent further increase in 16-19 year olds, and no cases occurring ≥20 years old. The results of univariable conditional logistic regression of signalment are presented in Table 1. The odds of being a case were significantly lower in the 0-1 year and 11-20 year age categories compared to those aged 2-10 years (P<0.001). The odds of being a case were significantly greater in native Scottish breeds (P<0.001) and significantly less in other pure breeds (P = 0.015) when compared with cross-breeds. Native Scottish breeds were located further north than other breed groups, with 59% located north of

OS northing 700000 [56°10'N] compared to <43% for other breeds. There were no differences in proportion of cases with respect to sex (P>0.60).

Meteorological variables relationships with each other and OS easting and northing

Average meteorological variables from 1990-2006 are presented in Figs 3a-f. The relationships between the meteorological variables are presented in Supplementary Item 1, with a high degree of correlation (both positive and negative) between all variables (P<0.001). Relationships between meteorological variables and OS easting and OS northing were in the main curvilinear (Supplementary Items 1 and 2).

Univariable conditional analysis of meteorological variables

The results of univariable conditional logistic regression of meteorological variables are presented in Table 2. There were lower maximum temperatures where cases were located compared to controls (average difference 0.08-0.13ºC) for all time periods studied. This difference was significant at the month of admission (P = 0.009) and the average of 3 and 12 months prior to admission (P<0.028). There were more frost days in cases compared to controls (average difference 0.05-0.2 days), this being statistically significant if 6 or more months were averaged (P = 0.019). More sun hours was observed for cases (average difference 0.01-0.05 h), but only statistically significant for the average of 6 months prior to admission (P = 0.016). There were no statistically significant associations between being a case and either rainfall or rain days, or minimum temperature (P>0.091).

Final multivariable conditional logistic models

OS northing, breed, age category and sex (P<0.25, Table 1) were entered into a *multivariable conditional* model. Models were then run for the 5 different average meteorological measurements based on the univariate results in Table 2 (P<0.25). The results of the final multivariable conditional logistic regression models of signalment are presented in Table 3. No meteorological variable remained in the models if OS northing was included. However, there was still an increased odds of being a case the further north a horse was located (P<0.001). Horses aged 11-20 were still significantly associated with lower odds compared to 2-10 year olds (P<0.001), but odds of being a case in <one year olds were no longer different (P<0.240). Native Scottish breeds were still more likely to be cases (P<0.001), and other pure breeds less likely compared to cross-breeds (P=0.018). In addition, stallions were now associated with lower odds of being a case (P = 0.018) compared to mares. This was due to a greater difference in the percentage of cases between mares and stallions aged 2-10 years (57% and 25%, respectively) compared to all mares and stallions (35% and 24%, respectively).

Given the OS northing results above, the 5 initial models were re-run with OS northing excluded. How far north a horse was located could be considered as not indicative of any aetiologically relevant parameter, and was more likely to be acting as a confounder for other environmental, meteorological or geochemical parameter(s). However, even with excluding OS northing the high degree of correlation between weather variables (Supplementary Item 1) made determining the final *multivariable conditional* models problematic, particularly between the 2 temperature measurements. It was therefore decided to select maximum temperature for entry into the *multivariable conditional* models as this was more consistently associated with significant *univariate conditional* results (Table 2). The confounding between maximum temperature and frost day meant that where appropriate, models with and without maximum temperature are also presented. The results of the final multivariable conditional logistic regression models of meteorological variables are presented in Table 3. Maximum temperature remained in all 5 models with the odds of being a case decreasing with higher temperature ($P<0.011$). In addition, the odds increased with sun hours ($P<0.009$) for all measurements apart from the average of 1990-2006. More frost days was associated with increased odds in the average of the 12 months prior to admission data ($P = 0.025$). If maximum temperature was excluded, then more frost days were associated with increased odds with the average of 6, and 12 months prior to admission data as well as the average for 1990-2006 data ($P<0.026$), and a slight decrease in the odds with increasing rainfall in the average of the 12 months prior to admission model ($P = 0.018$).

Discussion

This is the largest case-control study to examine signalment- and meteorological- risk factors for EGS [4-7; 9; 12; 14; 16-19]. Cases were predominantly from Eastern Scotland and within this region, the odds of a horse being a case significantly increased the further north the horse was located but was unaffected by how far east. However, rather than acting as a causal factor *per se*, the association of EGS with increased OS northing likely reflects an environmental, meteorological or geochemical risk factor that changes with OS northing [5; 19]. Owner address was considered a valid proxy of horse location, since a previous study estimated that 90% of horses are located within 10 km of their owners residence and 61% are kept at the same postcode [20].

Animals aged between 2-10 years old were significantly more likely to be EGS cases, compared to 11-20 years old, even when accounting for breed and sex. As with the present study, McCarthy *et al*. [4] found a non-linear relationship with age (horses aged 4-5 years were at maximum risk, with risk in older age groups declining progressively) in a premises matched study of 66 confirmed cases and 132 controls. In an earlier study within the same area of Scotland to the present study, Doxey *et al.* [5] found younger animals (<7 years old) were more susceptible. The association of EGS with particular age classes likely reflects different levels of protective passive and acquired immunity and/or tolerance to causal agents such as *Clostridium botulinum* type C [4; 6; 21].

We have identified an apparent increased proportion of native Scottish breeds that were referred because of EGS compared to other breeds. This study is the first study to demonstrate an apparent association between native Scottish breeds and an increased risk of EGS. In addition, there was decreased odds of being a case in the other pure breed horses compared to mixed breeds. One possibility is that we have referral bias in our data – native Scottish breeds may be less likely to be referred for non-EGS reasons for example. There is no evidence for this, but such biases cannot be ruled out. However it may be these results reflect inherent breed susceptibility or are influenced by other confounding management factors that were not tested in this study, such as an increased duration of grazing compared with other breeds. Previous analyses have identified significant associations between breed and EGS [4] and increased odds of EGS in heavy draught horses [6]. In contrast to the present study, Wood *et al.* [6] discarded this breed association from their final *multivariable conditional* models, due to its addition being inconsistent to model significance. Further focussed epidemiological investigations on native Scottish breeds, including reasons for admission to a referral hospital, are therefore warranted.

EGS was not significantly associated with sex in the *univariable conditional* analyses, consistent with previous findings [4; 12; 22]. However, in the *multivariable conditional* model, stallions had a lower risk of EGS compared to mares. This contrasts with findings of Wood *et al.* [6] that mares were less likely to develop EGS than males. The reasons for this conflicting result remain unclear, but may reflect different study designs or study populations or biases as outlined above.

Significant associations were found between EGS and a number of meteorological variables, even if the magnitude of the average differences were not large (Table 2); however, the results are further complicated by the high degree of correlation between variables and the lack of any associations if OS northing was included in the *multivariable conditional* models. The models excluding OS northing revealed that meteorology-related risk factors with an increased risk of EGS were: lower average minimum and maximum temperatures (0.08-0.13ºC); greater sun hours (0.01-0.05 h) and frost days (0.05-0.2 days).

While the average differences were not large, the overall association of the 455 EGS cases compared to the 910 controls with lower maximum and minimum temperatures appears to be consistent with previous reports that EGS commonly follows periods of cool weather [6; 14]. However, these previous studies reported a temporal association between EGS and cool weather, while the present study identified for the first time that horses on premises with lower *average* maximum and minimum and temperatures were at increased risk of EGS. The association of EGS and more frost days was not strong, and was only apparent for data taken over the last 6 or 12 months prior to a case, with no such associations with frost days more closely associated with the case and the average differences not large. In addition, the results are likely to be confounded by other meteorological variables. Nevertheless, the result was consistent with previously reported studies [13; 14]. EGS, equine laminitis and Mare Reproductive Loss Syndrome, have been associated with pasture grazed when there have been more frequent frosts [14; 23; 24]. Cooler weather could potentially predispose to EGS in several ways, but must do so only when acting in combination with other seasonal risk factors, otherwise EGS would be most common in winter. Cool weather may increase consumption of herbage containing the causal agent because it increases nutritional demands [25], or may enhance survival of soil bacteria [26] and fungi [27], which may contribute to disease development. The concentration of fructans in grasses may increase on cold bright days when photosynthesis rates are high but there is negligible plant growth [28]. Increased fructan levels can initiate changes in caecocolic microflora [29-31], which could potentially be a trigger factor for EGS. Pasture herbage growing at lower temperatures may have reduced bioavailability of potentially protective nutrients such as magnesium and antioxidants [32]. Additionally frost induces acute changes in plant metabolism, altering nitrate, sodium and potassium, and increasing amino acids and simple sugars [33; 34]. These changes may make the plants susceptible to colonisation by fungal organisms such as *Aspergillus spp* and bacterial species such as *Clostridium,* a currently favoured aetiological cause of EGS [35]. Such organisms are likely on frost damaged forages as they are present in manure and soil

[36-38].

There was no apparent association between rain days and EGS. Begg [13] suggested that wet weather followed by warm temperature resulted in numerous cases. However, rain days does not appear to be a key determinant of disease occurrence [39] and previous studies report that EGS commonly followed a 2 week period of predominantly dry weather [14] or dry weather followed by rain [6].

There was an association with more average sun hours over the 6 months prior to case and an increased risk of EGS. This is a novel finding, not previously identified with EGS [14]; however again this must be interpreted with caution given the magnitude of intergroup differences (average difference 0.01-0.05 hours). An average difference of 0.05 hours would translate into an average of 9 more hours of sunshine over 6 months, with the odds of being a case increasing by 1.62 for every sun hour. More sun hours may be associated with increased production of pasture fructans [28; 40] with increased consumption may lead to gastrointestinal disturbance as previously described. Alternatively, more sun hours may trigger a casual micro-organism on the pasture to multiply and/or produce toxins. Furthermore, animals may be more likely to be turned out for longer during increased sunshine periods, increasing potential exposure to causal toxin.

This study utilised the entire R(D)SVS database of EGS cases for 1st January 1990 to 1st June 2006. While a small proportion of chronic EGS cases were diagnosed solely on the basis of clinical signs and history, misclassification error is likely to be rare since Milne *et al.* [41] reported 100% accuracy of diagnosis of chronic EGS at the R(D)SVS using clinical examination alone. It is, however, acknowledged that the potential inclusion of controls from premises where EGS had previously occurred may introduce bias. The study used interpolated meteorological values, precluding evaluation of local meteorological conditions.

Inclusion of data from other regions of Great Britain would overcome the geographical bias of this study, and perhaps optimise identification of the potential influence of meteorological variables.

In conclusion, in Scotland, signalment-related risk factors associated with an increased risk of EGS were native Scottish breeds compared to cross-breeds and animals living on premises located further north within the study region. There was a decreased risk of EGS in animals aged 11-20 years old compared to animals 2-10 years old, non-native Scottish pure breeds compared to cross-breeds and stallions compared to mares. Meteorology-related risk factors associated with an increased risk of EGS were more sun hours and more frost days, while there was a decreased risk of EGS with higher average maximum temperature. This information will inform owners of high-risk animals, thereby allowing them to prioritise management strategies and assist future studies on the aetiology of EGS.

Authors' declaration of interests

No conflicts of interest have been declared.

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Author contributions

C.E.W., D.J.S., F.F.M., A.L. and B.C.McG contributed to the design of the study which was conducted by C.E.W.. The manuscript was drafted by D.J.S. and C.E.W. with input from F.F.M., A.L. and B.C.McG.. Statistical analysis and interpretation were by D.J.S..

Table Legends

Table 1: Univariable conditional logistic regression analysis of the associations between location, age group, breed and sex and equine grass sickness. ref - reference level; NA - no odds ratio possible as 0% cases in that age category; - not applicable, OR = odds ratio, CI = confidence interval

Table 2: Univariable conditional logistic regression analysis of meteorological variables associated with equine grass sickness. OR = odds ratio, CI = confidence interval

Table 3: Multivariable conditional logistic regression analysis of risk factors associated with equine grass sickness. (a) Variable left in model when Ordnance Survey northing included; (b) Meteorological variables left in model when Ordnance Survey northing excluded (other signalment variables still in final model and as for (a)). In addition, where appropriate, models with maximum temperature excluded (T_{max} exc) are presented. ref - reference level; NA - no odds ratio possible as 0% cases in that age category. OR = odds ratio, CI = confidence interval

Figure legends

Fig 1: Map of Scotland showing the location of equine grass sickness cases (purple) and controls (cream). Map source - 2001 Census Output Area Boundaries. Crown copyright 2003. Crown copyright material is reproduced with the permission of the Controller of HMSO. Dotted horizontal and vertical lines indicate OS Northing 890000 and Easting 240000 respectively.

Fig 2: The relationship between age of a horse and percentage of all horses at that age that were had equine grass sickness. Vertical dotted lines are the 95% exact binomial confidence intervals and the number in brackets is the total number of horses at that age (cases+controls).

Fig 3a-f: Maps of interpolated average annual meteorological variables for Scotland (1990-2006) (a) sun hours, (b) rain days, (c) rainfall (mm), (d) frost days, (e) minimum temperature and (f) maximum temperatures ($^{\circ}$C). The range of values for each meteorological variable are indicated by the shaded bar on each plot. Locations of equine grass sickness cases and controls are also shown on each figure.

Table 1

Factor	Group	Total	Cases			Controls		OR (95% CI)	P-value	
			N	(%)	Mean ± s.d.	N	Mean ± s.d.		Overall	Individual
Location	Easting (per 10 km) [a]	-	-	-	32.9 ± 3.53	-	32.6 ± 3.09	1.02 (0.98-1.06)	0.286	
	Northing (per 10 km) [b]	-	-	-	72.5 ± 6.98	-	68.9 ± 6.24	1.08 (1.06-1.10)	<0.001	
Age category	0-1	102	23	22.5	-	79	-	0.43 (0.27-0.70)	<0.001	<0.001
	2-10	915	374	41.0	-	539	-	ref		
	11-20	315	58	18.4	-	268	-	0.33 (0.24-0.45)		0.001
	>20	24	0	0.0	-	24	-	NA		NA
Breed	Cross-breeds	632	204	32.3	-	428	-	ref	<0.001	
	Native Scottish breeds	185	111	60.0	-	74	-	3.51 (2.43-5.07)		<0.001
	Other pure breeds	548	140	25.5	-	408	-	0.73 (0.57-0.94)		0.015
Sex	Female	594	204	34.3	-	390	-	ref	0.486	
	Male	771	251	32.6	-	520	-	0.92 (0.73-1.16)		
Sex group	Mare	556	197	35.4	-	359	-	ref	0.060	
	Filly	38	7	18.4	-	31	-	0.41 (0.18-0.95)		0.037
	Stallion	67	16	23.9	-	51	-	0.56 (0.31-1.02)		0.259
	Colt	53	15	28.3	-	38	-	0.70 (0.38-1.30)		0.057
	Gelding	651	220	33.8	-	431	-	0.92 (0.73-1.17)		0.516

[a] 21 OS easting west of 240000 excluded

[b] 13 OS northing north of 890000 excluded

Table 2

		Rain days (days/month with >1 mm)	Rainfall (mm/month)	Sun (hours/month)	Temperature - maximum (°C)	Temperature - minimum (°C)	Frost (days/month with frost)
Average for month of admission	Cases	11.52 ± 4.50	67.98 ± 42.72	4.85 ± 1.67	13.80 ± 3.60	5.84 ± 2.88	7.15 ± 6.29
	Controls	11.41 ± 4.59	67.89 ± 41.63	4.80 ± 1.66	13.93 ± 3.55	5.91 ± 2.86	7.10 ± 6.17
	OR (95% CI)	1.02 (0.97-1.08)	1.0001 (0.9954-1.0049)	1.23 (0.98-1.54)	**0.83 (0.73-0.95)**	0.88 (0.76-1.02)	1.02 (0.96-1.09)
	P value	0.363	0.951	0.074	**0.009**	0.092	0.564
Average for previous 3 months	Cases	11.66 ± 2.79	69.27 ± 30.43	4.34 ± 1.21	12.29 ± 3.14	4.74 ± 2.56	9.70 ± 5.30
	Controls	11.63 ± 2.60	69.01 ± 27.64	4.30 ± 1.19	12.40 ± 3.11	4.80 ± 2.54	9.64 ± 5.29
	OR (95% CI)	1.008 (0.948-1.072)	1.001 (0.995-1.006)	1.37 (0.99-1.90)	**0.84 (0.73-0.97)**	0.88 (0.75-1.03)	1.03 (0.95-1.10)
	P value	0.796	0.831	0.055	**0.019**	0.106	0.503
Average for previous 6 months	Cases	12.17 ± 2.27	75.04 ± 26.26	3.52 ± 0.94	10.55 ± 2.83	3.57 ± 2.29	12.69 ± 5.02
	Controls	12.21 ± 2.17	76.17 ± 25.21	3.48 ± 0.91	10.63 ± 2.82	3.64 ± 2.28	12.49 ± 4.96
	OR (95% CI)	0.99 (0.93-1.05)	0.998 (0.993-1.003)	**1.62 (1.10-2.40)**	0.87 (0.74-1.01)	0.88 (0.75-1.03)	**1.10 (1.02-1.18)**
	P value	0.723	0.389	**0.016**	0.069	0.114	**0.019**
Average for previous 12 months	Cases	12.29 ± 1.86	77.72 ± 21.38	3.56 ± 0.38	11.79 ± 0.97	4.71 ± 0.91	10.42 ± 1.76
	Controls	12.36 ± 1.74	79.20 ± 21.17	3.54 ± 0.34	11.88 ± 0.87	4.77 ± 0.82	10.22 ± 1.60
	OR (95% CI)	0.97 (0.90-1.04)	0.996 (0.991-1.002)	1.34 (0.88-2.04)	**0.84 (0.72-0.98)**	0.89 (0.76-1.04)	**1.15 (1.04-1.26)**
	P value	0.427	0.199	0.178	**0.028**	0.145	**0.005**
Average for 1990-2006	Cases	12.36 ± 1.53	78.10 ± 19.72	3.60 ± 0.25	11.95 ± 0.78	4.83 ± 0.77	10.14 ± 1.19
	Controls	12.42 ± 1.39	79.05 ± 18.51	3.59 ± 0.22	12.04 ± 0.69	4.89 ± 0.69	9.98 ± 0.94
	OR (95% CI)	0.97 (0.90-1.05)	0.997 (0.991-1.003)	1.22 (0.75-1.99)	0.87 (0.75-1.02)	0.92 (0.78-1.08)	**1.17 (1.05-1.31)**
	P value	0.475	0.385	0.416	0.083	0.294	**0.006**

Table 3

(a) Signalment	Group / variable	OR (95% CI)	P-values Overall	P-values Individual
Location	OS Northing (per 10 km)	1.08 (1.06-1.10)	<0.001	
Age category	0-1	0.33 (0.05-2.10)		0.240
	2-10	ref	<0.001	
	11-20	0.32 (0.22-0.45)		<0.001
	>20	NA		NA
Breed	Cross-breeds	ref	<0.001	
	Native Scottish breeds	3.56 (2.34-5.43)		<0.001
	Other pure breeds	0.71 (0.54-0.94)		0.018
Sex	Mare	ref	0.023	
	Filly	0.64 (0.08-5.10)		0.671
	Colt	1.92 (0.27-13.49)		0.516
	Stallion	0.43 (0.22-0.86)		0.018
	Gelding	1.07 (0.81-1.41)		0.652
(b) Meteorological + signalment (Exc OS Northing)				
Month of admission	Sun (hours)	1.44 (1.09-1.89)	0.009	
	Temperature $_{max}$	0.77 (0.66-0.91)	0.002	
Average previous 3 months	Sun (hours)	1.88 (1.25-2.81)	0.002	
	Temperature $_{max}$	0.76 (0.63-0.92)	0.004	
Average previous 6 months	Sun (hours)	2.48 (1.52-4.04)	<0.001	
	Temperature $_{max}$	0.73 (0.60-0.89)	0.001	
	T_{max} exc			
	Sun (hours)	2.04 (1.30-3.21)	0.002	
	Frost (days)	1.14 (1.04-1.24)	0.004	
Average previous 12 months	Sun (hours)	2.39 (1.38-4.14)	0.002	
	Temperature $_{max}$	0.76 (0.61-0.94)	0.010	
	Frost (days)	1.14 (1.02-1.28)	0.025	
	T_{max} exc			
	Rainfall (mm)	0.992 (0.985-0.999)	0.018	
	Frost (days)	1.18 (1.06-1.31)	0.003	
Average of 1990 - 2006	Temperature $_{max}$	0.82 (0.69-0.98)	0.028	
	T_{max} exc			
	Frost (days)	1.18 (1.04-1.34)	0.011	

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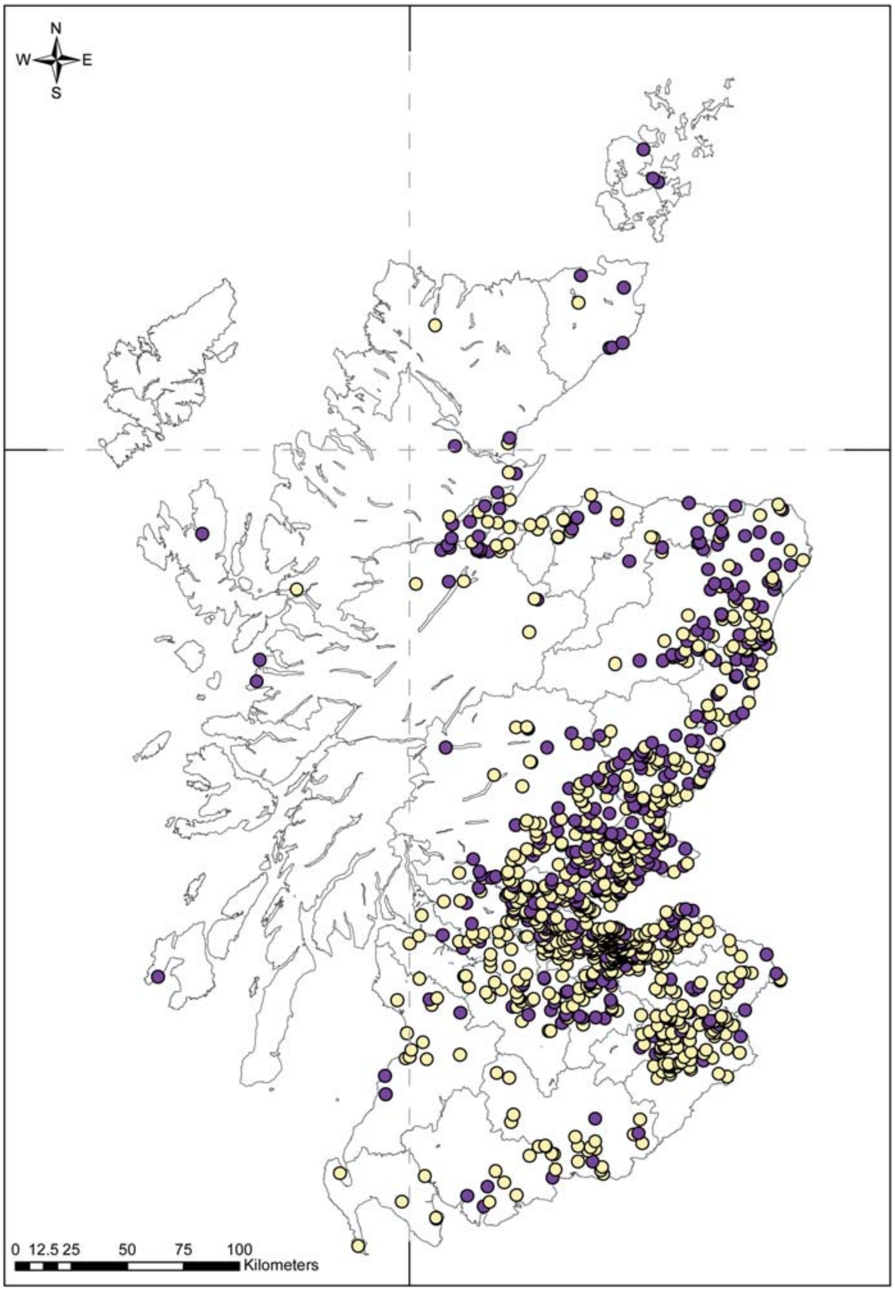
Supplementary Items

Supplementary Item 1 - Wylie EGS EVJ-GA-11-369R2 SupplementaryInfoText.doc

Supplementary Item 2 - Wylie EGS EVJ-GA-11-369R2 SupplementaryInfoTable.doc



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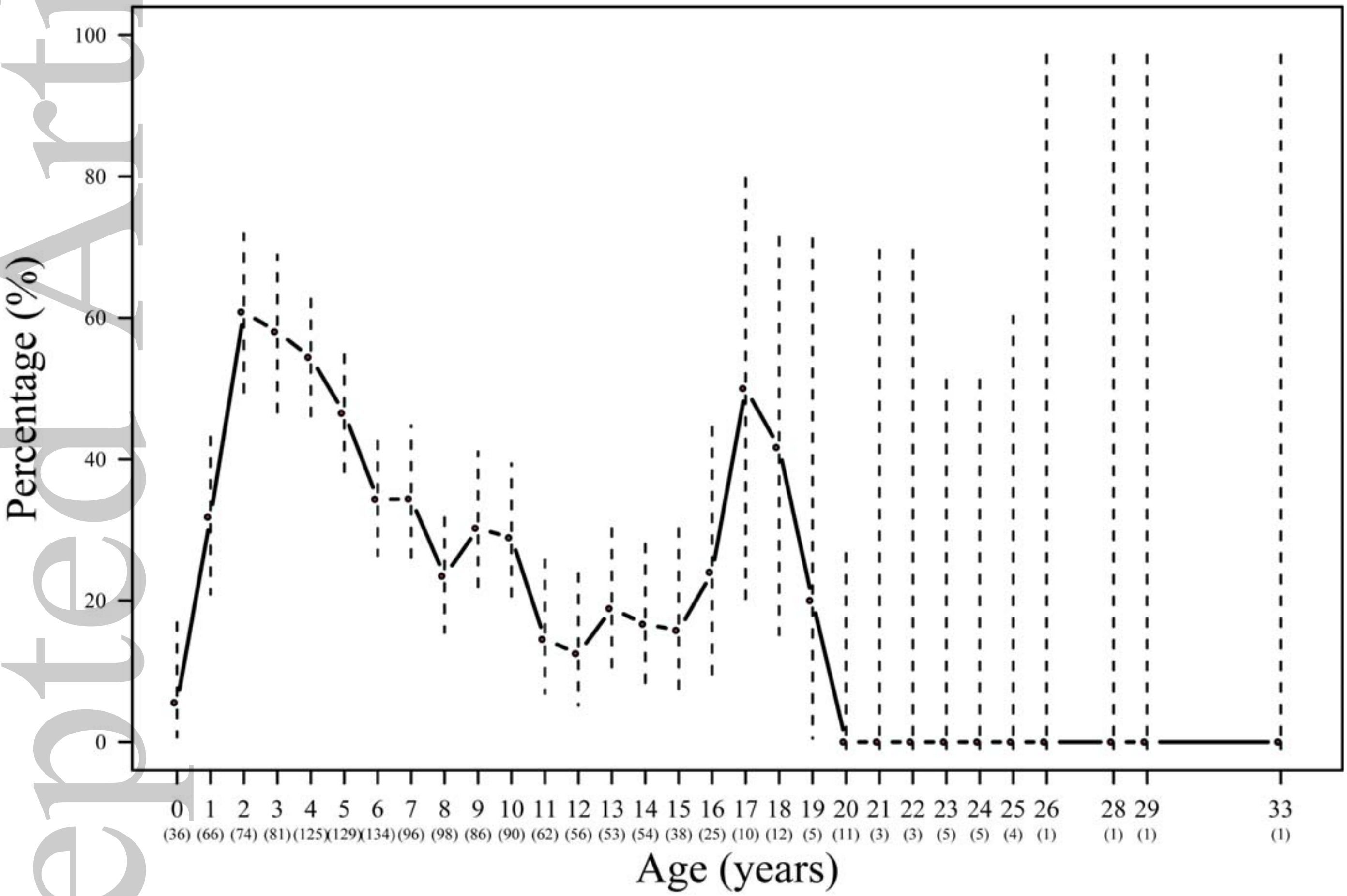
100
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Age (years)

Figure2.tif

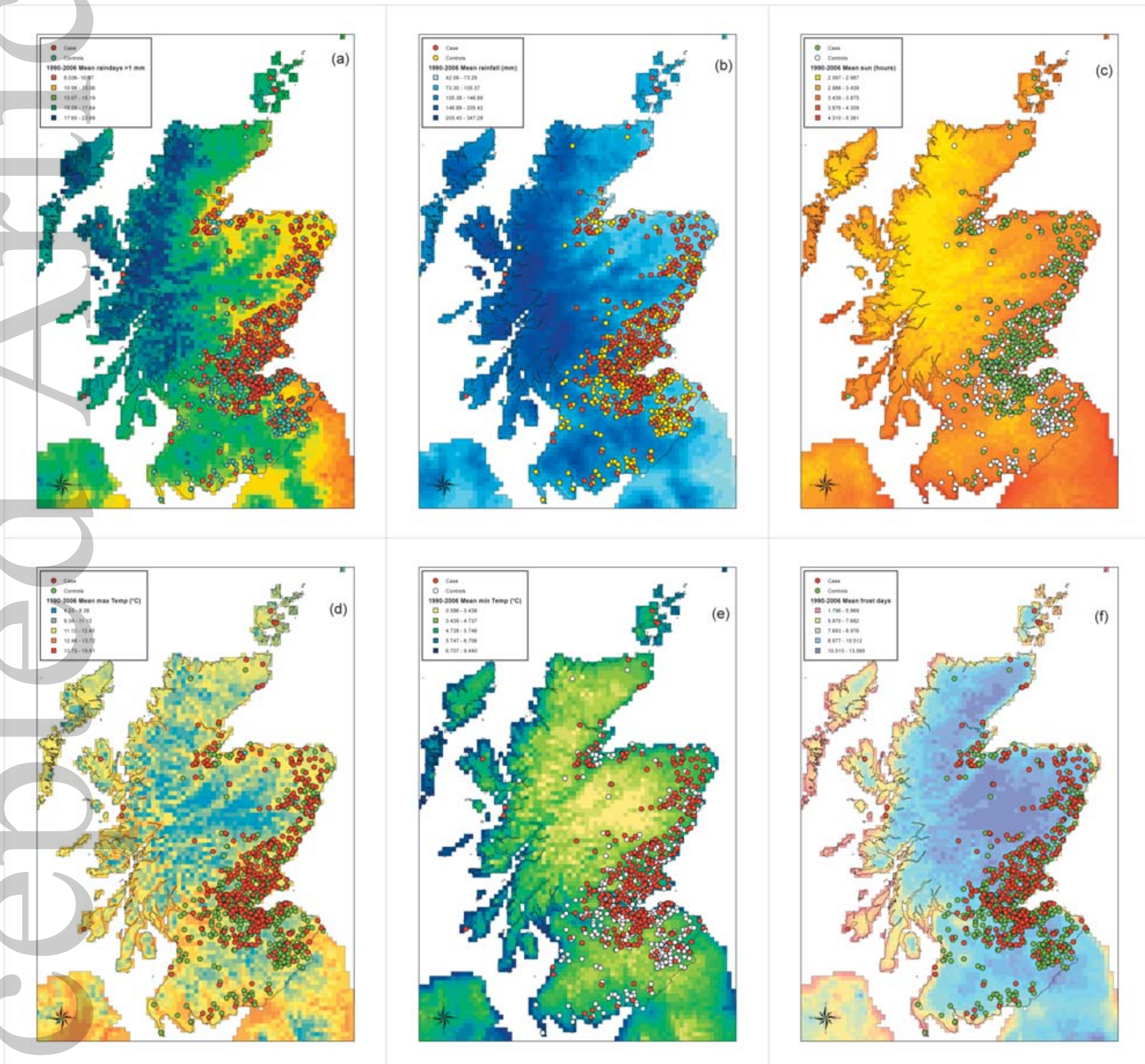
(a)
Case
Controls
1990-2006 Mean raindays >1 mm
(b)
Case
Controls
1990-2006 Mean rainfall (mm)
(c)
Case
Controls
1990-2006 Mean sun (hours)
(d)
Case
Controls
1990-2006 Mean max Temp (°C)
(e)
Case
Controls
1990-2006 Mean min Temp (°C)
(f)
Case
Controls
1990-2006 Mean frost days

Figure3.tif